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(33) Priority Country: US

(71) Applicant: MIDCON LABS, INC. [US/US]; 103 W. 12th Street, Lamar, MO 64759 (US).

(72) Inventors: NELSON, Ralph ; 5937 Ballentine, Shawnee, KS 66203 (US). SCHLINK, Gerald ; Rt. #1, Irwin, MI 64759 (US).

(74) Agents: LASSEN, Elizabeth et al.; Wegner & Bretschneider, P.O. Box 18218, Washington, DC 20036 (US).

(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), HU, IT (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent).

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(54) Title: CO-VACCINATION USING NON-O-CARBOHYDRATE SIDE-CHAIN GRAM-NEGATIVE BACTERIA PREPARATION

(57) Abstract

A composition for co-injection of an animal against a gram-negative pathogen which comprises an effective dose of a gram-negative type lipopolysaccharide devoid of O-carbohydrate side-chains and a vaccine derived from said pathogen. Methods of co-injection of an animal to protect the animal against gram-negative pathogens.

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AMENDED CLAIMS

[received by the International Bureau on 25 November 1987 (25.11.87); original claims 1 and 8 amended ; remaining claims unchanged (2 pages)]

1. A composition effective for co-injection of an animal to enhance the immune response of said animal against a gram-negative pathogen which comprises an effective dose of

a) bacterial lipopolysaccharide devoid of O-carbohydrate side-chains; and

b) a whole cell bacterin derived from said pathogen.

2. A composition as in claim 1, wherein said lipopolysaccharide is contained in cells of gram-negative bacteria.

3. A composition as in claim 2, wherein said cells are E. coli J5 or mutants thereof.

4. A composition of claim 1, wherein said pathogen is one or more selected from the group consisting of Pasteurella multocida, P. hemolytica, Escherichia coli, Bordetella bronchiseptica, Salmonella typhimurium, S. choleraesuis, S. dublin, Pseudomonas aeruginosa, Haemophilus pleuropneumoniae, H. parasuis, H. sommnus, Moraxella bovis, Treponema hyodysenteriae, Campylobacter sputurum, C. hyointestinalis, Leptospira canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae, L. pomoma, and L. bratislava.

5. A composition of claim 3, wherein said pathogen is one or more selected from the group consisting of Pasteurella multocida, P. hemolytica, Escherichia coli, Bordetella bronchiseptica, Salmonella typhimurium, S. choleraesuis, S. dublin, Pseudomonas aeruginosa, Haemophilus pleuropneumoniae, H. parasuis, H. sommnus, Moraxella bovis, Treponema hyodysenteriae, Campylobacter sputurum, C. hyointestinalis, Leptospira canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae, L. pomoma, and L. bratislava.

6. A composition as in claim 2, wherein said cells are concentrated to about 5 to about 50 percent by volume.

7. A composition as in claim 3, wherein said cells are concentrated to about 2-3 x $10^{10}$ cfu/ml.

8. Process of enhancing the immune response of an animal susceptible to infection by a gram-negative pathogen which comprises co-injection of an effective dose of

a) bacterial lipopolysaccharide devoid of O-carbohydrate side-chains; and

b) a whole cell bacterin derived from said pathogen.

9. A process as in claim 8, wherein said lipopolysaccharide is contained in cells of gram-negative bacteria.

10. A process as in claim 9, wherein said cells are E. coli J5 or mutants thereof.

11. A process of claim 8, wherein said pathogen is one or more selected from the group consisting of Pasteurella multocida, P. hemolytica, Escherichia coli, Bordetella bronchiseptica, Salmonella typhimurium, S. choleraesuis, S. dublin, Pseudomonas aeruginosa, Haemophilus pleuropneumoniae, H. parasuis, H. sommnus, Moraxella bovis, Treponema hyodysenteriae, Campylobacter sputurum, C. hyointestinalis, Leptospira canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae, L. pomoma, and L. bratislava.

12. A process of claim 10, wherein said pathogen is one or more selected from the group consisting of Pasteurella multocida, P. hemolytica, Escherichia coli, Bordetella bronchiseptica, Salmonella typhimurium, S. choleraesuis, S. dublin, Pseudomonas aeruginosa, Haemophilus pleuropneumoniae, H. parasuis, H. sommnus, Moraxella bovis, Treponema hyodysenteriae, Campylobacter sputurum, C. hyointestinalis, Leptospira canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae, L. pomoma, and L. bratislava.

13. A process as in claim 9, wherein said cells are concentrated to about 5 to about 50 percent by volume.

14. A process as in claim 10, wherein said cells are concentrated to about 2-3 x $10^{10}$ cfu/ml.

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**Published**
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(57) Abstract

A composition for co-injection of an animal against a gram-negative pathogen which comprises an effective dose of a gram-negative type lipopolysaccharide devoid of O-carbohydrate side-chains and a vaccine derived from said pathogen. Methods of co-injection of an animal to protect the animal against gram-negative pathogens.

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# CO-VACCINATION USING NON-O-CARBOHYDRATE SIDE-CHAIN GRAM-NEGATIVE BACTERIA PREPARATION

## Field of the Invention

This invention relates to an improved composition for co-vaccination of animals, including mammals and birds, against gram-negative organisms and the diseases caused thereby. More particularly, the invention concerns a co-vaccine that employs a bacterial lipopolysaccharide (LPS) fraction devoid of O-carbohydrate side-chains, exemplified by E. coli J5 and mutants thereof, with a bacterin directed to one or more gram-negative organisms for the immunological protection of an animal against gram-negative organisms, and the diseases caused by these organisms.

## Background of the Invention

Gram-negative bacteria have similar LPS structures. Some mutant gram-negative bacterial strains lack the O-carbohydrate side-chains normally associated with gram-negative bacteria. These mutant organisms lack pili and outer antigens which are normally associated with the LPS membrane leaving LPS and other core antigens exposed.

Escherichia coli strain J5 is a well known example of a genetically stable gram-negative bacterial species having LPS and other core antigens exposed. Other gram-negative bacteria of different species, such as Salmonella enteritidis, ATCC No. 53000, described in European Patent Application 0158282, also lack the O-carbohydrate side-chains (also known as "K antigens"). The European Patent Application teaches a method of preparing non-O-carbohydrate side-chain gram-negative bacteria.

## Summary of the Invention

In accordance with the present invention, a co-vaccine suitable for administration against gram-negative organisms which contains an effective dose of a bacterial lipopolysaccharide devoid of O-carbohydrate side-chains, bacterins of one or more gram-negative organisms, and

optionally a pharmaceutically acceptable carrier, is disclosed. Administration of the co-vaccine is achieved by the co-injection of the bacterial lipopolysaccharide devoid of O-carbohydrate side-chains and the bacterins.

Also disclosed is a method of enhancing the immune response, and thus protecting an animal against diseases caused by gram-negative organisms by co-injecting the animal with an effective amount of bacterial lipopolysaccharide (LPS) devoid of O-carbohydrate side-chains in combination with bacterins of one or more gram-negative organisms.

Detailed Description of the Invention

The present invention relates to the co-administration of an effective amount of a bacterial lipopolysaccharide devoid of O-carbohydrate side-chains in conjunction with a vaccine specifically directed to each gram-negative organism to which immunization is desired. It has been found that bacterial lipopolysaccharide devoid of O-carbohydrate side-chains is an effective immunomodulator when used in conjunction with gram-negative bacterins.

It has been found that the process of co-injection of bacterial LPS devoid of O-carbohydrate side-chains in combination with gram-negative bacteria provides an advantage over gram-negative bacterin preparations used alone. The process of co-injection encompasses contemporaneous administration.

The co-vaccine of the present invention may optionally be administered in admixture with a pharmacologically acceptable carrier prior to administration to an animal. Pharmacologically acceptable carriers for the invention are those usually employed in vaccines such as aqueous and oil based carriers and includes slow release antigen/adjuvant combinations. Exemplary of components in such carriers are saline, aluminum hydroxide gel, and carboxypolymethylene.

A sourc of bacterial lipopolysaccharide devoid of O-carbohydrate side-chains is required. E. coli strain J5, or mutants thereof, is exemplary of a gram-negative organism having exposed LPS and other core antigens. E. coli strain J5 whole cells are a preferred source of bacterial lipopolysaccharide devoid of O-carbohydrate side-chains.

E. coli strain J5, i.e., ATCC No. 39355, can be cultivated in suitable growth media such as brain heart infusion or tryptic soy broth. Either enriched or minimal nutrient media can be used and the culture may be grown in glass containers or fermentors. A preferred medium is Trypticase Soy Broth (Difco Laboratories, Detroit, MI). Frozen or lyophilized J5 cultures can be used to inoculate the media.

The size of an inoculum should not be less than 0.2 percent v/v of the total volume. Growth of the organism is monitored by utilization of sugars, change in pH units and a change in the absorbance of the culture.

Gram-negative bacterial cells devoid of O-carbohydrate side-chains and bacterins of the present invention may be live or inactivated, and may be used in any combination thereof. A preferred source of bacterial lipopolysaccharide devoid of O-carbohydrate side-chains is inactivated cells. It is also preferred to use inactivated vaccines.

Gram-negative bacterial cells devoid of O-carbohydrate side-chains can be inactivated by boiling or treatment with anti-bacterial agents such as formaldehyde (0.2 percent v/v), beta-propriolactone, or antibiotics. The preferred method to inactivate the cells is with formaldehyde. The cell culture can then be optionally concentrated and/or washed to remove media components. Washed and concentrated cells devoid of O-carbohydrate side-chains are preferred.

The cell culture is typically concentrated from 5-50 percent by volume. One method to concentrate cells is the hollow-fiber method (Amicon Corporation, Danvers, MA). This

method utilizes a hollow-fiber containing cartridge which includes a matrix of fibers through which the sample is pumped.

The preferred mode employs E. coli J5 cells, washed and concentrated to 2-3 x $10^{10}$ cfu/ml as determined optically. This corresponds to an approximate twenty-fold dilution of a J5 cell culture.

Bacterins of various types of gram-negative organisms are useful in the present invention. Specific examples of gram-negative bacterin strains are the following:

Pasteurella multocida, P. hemolytica, Escherichia coli, Bordetella bronchiseptica, Salmonella typhimurium, S. choleraesuis, S. dublin, Pseudomonas aeruginosa, Haemophilus pleuropneumoniae, H. parasuis, H. sommnus, Moraxella bovis, Treponema hyodysenteriae, Campylobacter sputurum, C. hyointestinalis, , Leptospira canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae, L. pomoma, and L. bratislava.

Bacterins useful in the present invention are prepared according to techniques known per se.

In the preparation of the compositions of the invention, the bacterins are preferably thoroughly mixed with the bacterial lipopolysaccharide devoid of O-carbohydrate side-chains. The admixture of the bacterin or bacterins with the bacterial lipopolysaccharide devoid of O-carbohydrate side-chains can occur during the formulation of the bacterin or after the bacterin itself has been prepared.

The bacterial lipopolysaccharide devoid of O-carbohydrates and bacterin is co-administered either undiluted or diluted with a pharmacological saline solution. Significant favorable results have been obtained with dilution values of up to about 1:25 with a number of pathogens tested. In numerous experiments undiluted or diluted solutions of about 1:5 have given very favorable results. Each milliliter of

vaccine preparation preferably contains 6 - 600 x $10^8$ cfu/ml of E. coli.

The co-vaccine composition can be used to enhance the immune response, and thus protect animals prior to infection of the animals with the gram-negative pathogen for which the inoculation is prepared. The co-vaccine can also be used to stimulate the immune response, and thus protect animals currently infected with the gram-negative pathogen for which the inoculation is prepared.

This invention can be used with animals having an antigen/antibody immune response system. Specific animals in which the invention can be used include such domestic mammals as cattle, sheep, goats, pigs, dogs, cats, and horses as well as poultry animals. An approximate typical dose is .5 - 1.5 ml for poultry, 1.0 - 3 ml for pigs and 2 - 4 ml for cattle.

The following Examples are used to illustrate the invention further but should not be deemed to limit it in scope.

Example 1 - Production of J5 Bacterin

E. coli J5 Bacterin is produced by inoculating media with actively growing seed (ATCC No. 39355). During the growth phase, the temperature is kept at 37°C ± 2°C and the pH is held constant at 7.0-7.3. The growth is monitored and maintained at a pH of 7.0-7.3 by the addition of 5 N sodium hydroxide. Dextrose is added as a sterile 50% solution to obtain maximum growth. At the end of the growth period the bacterin is inactivated with formaldehyde. After inactivation, tests are run to keep the free formaldehyde level below 0.2%.

Example 2 - J5 and Pasteurella Multocida

An E. coli J5 culture is grown and inactivated with formaldehyde as in Example 1. It is then concentrated to approximately 5-10% of its original volume and washed with 3 volumes of physiological saline solution.

The inactivat d, concentrated E. coli J5 Bacterin is then well combined with Midcon Labs' Pasteurella Multocida (PM) bacterin in the following proportions:

97.5 ml. PM Bacterin (25% solution)
2.5 ml. J5

95.0 ml. PM Bacterin (50% solution)
5.0 ml. J5

The vaccine preparations are administered subcutaneously or intramuscularly, undiluted or in a 1:5 dilution. The diluent is sterile phosphate buffered saline solution.

The results of comparative tests of the Midcon Labs' PM bacterin, E. coli J5 bacterin, and a combination of E. coli J5 bacterin with the PM bacterin appear in Table 1. USDA Pasteurella multocida (PM) Standard Reference Bacterin, IRP 248 and PM Challenge Culture, IRP 255 is used in the testing as per USDA test protocols.

TABLE 1

| DILUTION | USDA PM STANDARD | MIDCON PM STANDARD | MIDCON PM WITH 25% J5 | MIDCON PM WITH 50% J5 | 25% J5 ONLY | UNVACCINATED CONTROLS |
|---|---|---|---|---|---|---|
| A. UNDILUTED | #101* 12/20* | #201 11/20 | #301 17/19 | #401 13/19 | #501 4/20 | #601 0/20 |
| B. 1:5 | #102 1/20 | #202 1/20 | #302 4/20 | #402 1/20 | #502 0/20 | #602 X |

* No. Survivors/No. Challenged
* Cage No.
X No Mice in This Group

Example 3 - J5 and Salmonella Choleraesuis

A vaccine is prepared with E. coli J5 bacterin and Midcon Labs' Salmonella choleraesuis Bacterin, as in the procedures of Example 2.

The comparative test results of the use of either bacterin alone and the co-vaccine preparation of E. coli J5 bacterin and Salmonella choleraesuis bacterin appear in Table 2. The challenge culture is Salmonella choleraesuis, USDA IRP 224.

Table 2[1]

| DILUTION | SALMONELLA CHOLERAESUIS BACTERIN | SALMONELLA CHOLERAESUIS W/25% J-5 | SALMONELLA CHOLERAESUIS W/50% J-5 | 25% J-5 ONLY | 50% J-5 ONLY | UNVACCINATED CONTROLS |
|---|---|---|---|---|---|---|
| UNDILUTED | #201+ 11/20* | #301 15/20 | #401 12/20 | #501 3/20 | #601 4/20 | #701 0/20 |
| 1:5 | #202 4/20 | #302 10/20 | #402 5/20 | #502 1/20 | #602 1/20 | #702 X |

* No. Survivors/No. Challenged
\+ Cage No.
X No Mice in This Group

[1] No USDA standard bacterin available.

Example 4 - J5 and E. coli

A vaccine is prepared with E. coli J5 bacterin and E. coli Bacterin Sero Type 987p, as in the procedures of Example 2.

Table 3 shows the results of tests of the use of either bacterin alone and the co-vaccine preparation of E. coli J5 bacterin and E. coli bacterin. A further dilution of the vaccine is tested at 1:25 as well.

Table 3[1]

| DILUTION | E COLI BACTERIN | E COLI W/25% J-5 | E COLI W/50% J-5 | 25% J-5 ONLY | 50% J-5 ONLY | UNVACCINATED CONTROLS |
|---|---|---|---|---|---|---|
| UNDILUTED | #201* 12/20* | #301 20/20 | #401 20/20 | #501 14/20 | #601 13/20 | #701 0/20 |
| 1:5 | #202 6/20 | #302 20/20 | #402 18/20 | #502 8/20 | #602 10/20 | #702 X |
| 1:25 | #203 2/20 | #303 8/20 | #403 5/20 | #503 2/20 | #603 3/20 | #703 X |

* No. Survivors/No. Challenged
* Cage No.
X No Mice in This Group

[1] No USDA standard available.

Example 5

8 ml of Salmonella typhimurium Standard Reference Bacterin NVSL #81 IRP STB Serial 1 is mixed with 2 ml of saline, undiluted E. coli J5, E. coli J5 diluted 1:10, E. coli J5 diluted 1:100 and E. coli J5 diluted 1:1000. The admixture is then further diluted 1:5 and 1:25 with saline.

20 8 week old White Swiss Webster mice from SASCO, Omaha, NE are used for each dilution. The mice are vaccinated with 0.1 ml IP twice 2 weeks apart. 14 days after the second vaccination, the mice are challenged with 0.25 ml of a $10^4$ dilution of S. typhimurium.

Table 4 shows the results of the experiment S. typhimurium bacterin alone or in combination with E. coli J5 at various dilutions.

Table 4

| VACCINE | Dilution of S. typhimurium/E. coli J5 Bacterin Tested 1:5 | 1:25 |
|---|---|---|
| J-5 Undiluted | 2/20* | 4/20 |
| J-5 1:10 | 1/20 | 4/20 |
| J-5 1:100 | 3/17 | 6/20 |
| J-5 1:1000 | 2/20 | 9/20 |
| No J-5 | 2/18 | 6/14 |

* No. of Dead/No. Challenged

Example 6

8 ml of Midcon Labs' Pasteurella multocida is mixed with 2 ml of phosphate buffered saline, undiluted E. coli J5, E. coli J5 diluted 1:10, E. coli J5 diluted 1:100. The admixture is then either administered or further diluted to 1:5 with phosphate buffered saline.

Twenty six-week-old White Swiss Webster mice from SASCO, Omaha, Nebraska are used for each dilution. The mice are vaccinated with 0.1 ml IP and are challenged 14 days after the vaccination with 0.20 ml of a $10^6$ dilution of the challenge strain (USDA strain 169).

Table 5 shows the results of the experiment using bacterin alone or in combination with E. coli J5 at various dilutions.

Table 5

| Dilution of P. Multocida/E. coli J5 Bacterin Tested | | |
|---|---|---|
| VACCINE | UNDILUTED | 1:5 |
| J-5 Undiluted | 4/20* | 11/20 |
| J-5 1:10 | 8/20 | 17/20 |
| J-5 1:100 | 6/20 | 14/20 |
| No J-5 | 10/20 | 12/20 |

* No. of dead/No. challenged.

Example 7

A vaccine is prepared with E. coli J5 bacterin and Midcon Labs' Moraxella bovis (MB) as in the procedures of Example 2.

Cattle were vaccinated twice with either bacterin alone or the co-vaccine preparation of E. coli J5 bacterin and Moraxella bovis bacterin. The time lapse between the first and second vaccinations was 21 days. 25 days after the second vaccination the cattle were challenged with Moraxella bovis. The challenged cattle were examined 7, 15, 19, 29 and 40 days after challenge for visible gross ocular lesions.

Table 6 shows the results of the challenge testing.

TABLE 6

| DAYS AFTER CHALLENGE | MIDCON MB STANDARD | MIDCON MB WITH 25% J5 | MIDCON MB WITH 50% J5 | UNVAC-CINATED CONTROLS |
|---|---|---|---|---|
| 7 | 114/121* | 111/111 | 89/90 | 73/87 |
| 15 | 113/121 | 111/111 | 89/90 | 64/87 |
| 19 | 111/121 | 109/109+ | 86/90 | 50/86^ |
| 29 | 110/121 | 109/109 | 84/90 | 39/86 |
| 40 | 110/121 | 109/109 | 84/90 | 37/86 |

* No. of Symptom Free Animals/ No. Challenged.
\+ Two calves lost to an electrical storm.
^ One infected calf lost to an electrical storm.

WHAT IS CLAIMED IS:

1. A composition effective for co-injection of an animal to enhance the immune response of said animal against a gram-negative pathogen which comprises an effective dose of

a) bacterial lipopolysaccharide devoid of O-carbohydrate side-chains; and

b) a vaccine derived from said pathogen.

2. A composition as in claim 1, wherein said lipopolysaccharide is contained in cells of gram-negative bacteria.

3. A composition as in claim 2, wherein said cells are E. coli J5 or mutants thereof.

4. A composition of claim 1, wherein said pathogen is one or more selected from the group consisting of Pasteurella multocida, P. hemolytica, Escherichia coli, Bordetella bronchiseptica, Salmonella typhimurium, S. choleraesuis, S. dublin, Pseudomonas aeruginosa, Haemophilus pleuropneumoniae, H. parasuis, H. sommnus, Moraxella bovis, Treponema hyodysenteriae, Campylobacter sputurum, C. hyointestinalis, Leptospira canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae, L. pomoma, and L. bratislava.

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6. A composition as in claim 2, wherein said cells are concentrated to about 5 to about 50 percent by volume.

7. A composition as in claim 3, wherein said cells are concentrated to about 2-3 x $10^{10}$ cfu/ml.

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10. A process as in claim 9, wherein said cells are E. coli J5 or mutants thereof.

11. A process of claim 8, wherein said pathogen is one or more selected from the group consisting of Pasteurella multocida, P. hemolytica, Escherichia coli, Bordetella bronchiseptica, Salmonella typhimurium, S. choleraesuis, S. dublin, Pseudomonas aeruginosa, Haemophilus pleuropneumoniae, H. parasuis, H. sommnus, Moraxella bovis, Treponema hyodysenteriae, Campylobacter sputurum, C. hyointestinalis, Leptospira canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae, L. pomoma, and L. bratislava.

12. A process of claim 10, wherein said pathogen is one or more selected from the group consisting of Pasteurella multocida, P. hemolytica, Escherichia coli, Bordetella bronchiseptica, Salmonella typhimurium, S. choleraesuis, S. dublin, Pseudomonas aeruginosa, Haemophilus pleuropneumoniae, H. parasuis, H. sommnus, Moraxella bovis, Treponema hyodysenteriae, Campylobacter sputurum, C. hyointestinalis, Leptospira canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae, L. pomoma, and L. bratislava.

13. A process as in claim 9, wherein said cells are concentrated to about 5 to about 50 percent by volume.

14. A process as in claim 10, wherein said cells are concentrated to about 2-3 x $10^{10}$ cfu/ml.

15. A process of claim 10, wherein the animal is selected from the group consisting of cattle, pigs, horses, dogs, cats, sheep, goats and poultry animals.

16. A process as in claim 10, wherein the co-vaccine which also comprises a pharmacologically acceptable carrier.

17. A process as in claim 10, wherein said pharmacologically acceptable carrier is selected from the group consisting of aqueous or oil based carriers.

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US 87/01222

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: A 61 K 39/116; 37/20; 39/108; 39/39; // A 61 K 39/02; 39/102; 39/10; 39/112; 39/106; 39/104

## II. FIELDS SEARCHED

Minimum Documentation Searched [7]

| Classification System | Classification Symbols |
|---|---|
| IPC[4] | A 61 K |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

## III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

| Category [10] | Citation of Document, [11] with indication, where appropriate, of the relevant passages [12] | Relevant to Claim No. [13] |
|---|---|---|
| X | EP, A, 0089283 (MERCK) 21 September 1983 see page 2, lines 22-30; page 3, lines 1,2; page 22, lines 11-31; page 23, lines 9-31 -- | 1-7 |
| X | EP, A, 0158282 (THE CURATORS OF THE UNIVERSITY OF MISSOURI) 16 October 1985 see page 5, lines 28-38; page 6, lines 1-4, 14-26; page 7, lines 13-27; pages 8-11; page 13; page 14, lines 34-36; page 15, lines 1,2; claims cited in the application -- | 1-7 |
| X | GB, A, 2076287 (RAMOT UNIVERSITY) 2 December 1981 see page 1, lines 44-65; page 2, lines 1-5; page 4, lines 11-38; claims -- | 1-7 |
| X | Biological Abstracts, volume 74, no. 6, ./. | |

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

## IV. CERTIFICATION

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
|---|---|
| 24th August 1987 | 25 SEP 1987 |
| **International Searching Authority** | **Signature of Authorized Officer** |
| EUROPEAN PATENT OFFICE | L. ROSSI |

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FR M THE SECOND HEET)

| Category * | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No |
|---|---|---|
| | 1982, (Philadelphia, PA., US), M.I. Marks et al.: "Induction of immunity against lethal Haemophilus influenzae type b infection by Escherichia coli core lipopoly-saccharide", see page 4098, abstract 39459, J Clin Invest 69(4): 742-749 | 1-7 |
| X | The Lancet, volume II, 13 July 1985, The Lancet Ltd, J.-D. Baumgartner et al.: "Prevention of gram-negative shock and death in surgical patients by antibody to endotoxin core glyco-lipid", pages 59-63 see page 59, column 2, lines 3-20; page 62, column 2, lines 14-49; page 63, column 1, lines 1-27 | 1-7 |
| X | Infection and Immunity, volume 45, no. 3, September 1984, American Society for Microbiology, L.M. Mutharia et al.: "Monoclonal antibodies specific for Escherichia coli J5 lipopolysaccharide: cross-reaction with other gram-negative bacterial species", pages 631-636 see page 631, column 1, lines 1-34; column 2, lines 1-13; page 633, table I; page 635, "Discussion"; page 636 | 1-7 |
| X | Infection and Immunity, volume 46, no. 3, December 1984, American Society for Microbiology, M.J. Nelles et al.: "Mouse mono-clonal antibodies reactive with J5 lipopolysaccharide exhibit extensive serological cross-reactivity with a variety of gram-negative bacteria", pages 677-681 see the whole document | 1-7 |
| X | Biological Abstracts, volume 82, no. 9, 1986, (Philadelphia, PA., US), B.W. Fenwick et al.: "Mechanisms involved in protection by immunization against core lipopolysaccharides of Escherichia coli J5 from lethal Haemophilus pleuropneumoniae infections in swine", see page AB-466, abstract 82938, Infect Immun 53(2): 298-304, 1986 | 1-7 |

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FR M THE SECOND SHEET)

| Category * | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No |
|---|---|---|
| Y | US, A, 4455142 (MARTINS) 19 Jun 1984 see column 3, lines 41-68; column 4, lines 21-26, 61-65; examples 3,4 -- | 1-7 |
| Y | US, A, 4016253 (SWITZER) 5 April 1977 see the whole document -- | 1-7 |
| Y | US, A, 4469672 (HARRIS) 4 September 1984 see the whole document -- | 1-7 |
| Y | US, A, 4167560 (WOHLER) 11 September 1979 see column 7, lines 18-25 -- | 1-7 |
| Y | WO, A, 80/02113 (DORDISK DROGE & KEMIKALIE) 16 October 1980 see the whole document -- | 1-7 |
| Y | GB, A, 1085956 (SMITH) 4 October 1967 see the whole document | 1-7 |

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FURTHER INFORMATIO[redacted]TINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 8-17, because they relate to subject matter not required to be searched by this Authority, namely:

See PCT, Rule 39.1(iv) Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/US 87/01222 (SA 17372)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 15/09/87

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
|---|---|---|---|---|
| EP-A- 0089283 | 21/09/83 | JP-A- | 58167517 | 03/10/83 |
| | | US-A- | 4428931 | 31/01/84 |
| | | US-A- | 4488991 | 18/12/84 |
| EP-A- 0158282 | 16/10/85 | AU-A- | 4076785 | 10/10/85 |
| | | JP-A- | 61000021 | 06/01/86 |
| GB-A- 2076287 | 02/12/81 | NL-A- | 8101349 | 16/10/81 |
| | | FR-A,B | 2481928 | 13/11/81 |
| | | DE-A- | 3110801 | 07/01/82 |
| | | US-A- | 4404186 | 13/09/83 |
| US-A- 4455142 | 19/06/84 | None | | |
| US-A- 4016253 | 05/04/77 | None | | |
| US-A- 4469672 | 04/09/84 | None | | |
| US-A- 4167560 | 11/09/79 | None | | |
| WO-A- 8002113 | 16/10/80 | BE-A- | 882619 | 03/10/80 |
| | | EP-A- | 0026209 | 08/04/81 |
| | | GB-A,B | 2057882 | 08/04/81 |
| | | SE-A- | 8008500 | 03/12/80 |
| | | NL-T- | 8020132 | 30/01/81 |
| GB-A- 1085956 | | None | | |